

REMARKS

Claims 1 to 28 are pending in this application. No amendment to the claims is made in this Supplemental Preliminary Amendment.

Minor specification amendments are made in two places by this Supplemental Preliminary Amendment. In the position corresponding to page 2, line 1, of the original specification, the word “lung” is deleted, thereby completing the amendment of “3T3 mouse lung fibroblast” to – 3T3 mouse embryo fibroblast– throughout the specification. The second amendment is to correct “mg.ml”, which was a typographical error in the Preliminary Amendment of August 28, 2001, to the original –mg/ml–.

Applicants believe that the amendment of “3T3 mouse lung fibroblast” to –3T3 mouse embryo fibroblast– does not constitute new matter in the specification. The 3T3 cell line is well known in the art, but was characterized as a “mouse lung fibroblast” rather than a “mouse embryo fibroblast” in the original specification. This mischaracterization does not affect the description or enablement of the present invention, since the 3T3 cell line is well known and generally available, and the original phrase “3T3 mouse lung fibroblast” would be readily understood by one of ordinary skill in the art to refer to the generally available 3T3 cell line.

Nobutaka YAMAMOTO et. al.
Serial No. **09/718,388**

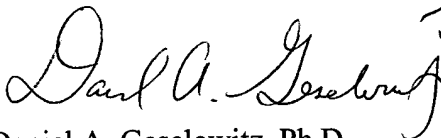
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Group Art Uni: **1636**

A prompt and favorable action on the merits is earnestly solicited.

In the event that any fees are due in connection with this paper, please charge our Deposit
Account No. 01-2340.

Respectfully submitted,

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Attachment: Version With Markings to Show Changes Made

Q:\FLOATERS\DAG\001554 Draft Supplemental preliiminary Amendment

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Please amend the paragraph beginning at line 23 of Page 1 and continuing to Page 2, as follows:

Conventionally, two methods have widely been employed for culturing epithelial cell, particularly epidermal cell (or which is called epidermal keratinocyte). One utilizes sterilized 3T3 mouse embryo fibroblast, i.e., viable 3T3 mouse ~~tung~~ embryo fibroblast from which division and proliferation potencies have been deleted by irradiating, for example, γ ray or by adding an agent such as mitomycin C, as feeder layer (such as the feeder layer culture method described in James G. Rheinwald and Howard Green. Cell 6: 331-344. Serial Cultivation of Strains of Human Epidermal Keratinocytes: the Formation of Keratinizing Colonies from Single Cells). The other utilizes serum-free medium such as MCDB153 instead of feeder layer.

Please amend the paragraph beginning at line 11 of Page 8, and continuing to Page 9, as follows:

Fibroblasts may be used those derived from mammals such as mouse, human, rat, hamster and rabbit. Preferably, 3T3 mouse embryo fibroblast, which is commonly used in conventional feeder layer culture methods, may be used. The condition for inoculating and

culturing cell is not particularly limited, and any standard condition may be used. For example, fibroblasts grown in a culture vessel may be separated by treating with trypsin solution (which was prepared by dissolving trypsin (0.25 weight/volume %) in a solution of 0.206 ~~mg/ml~~ mg/ml ethylenediamine-tetraacetic acid (EDTA) in phosphate buffer). The separated fibroblasts were suspended in a medium supplemented with 5 to 10% fetal bovine serum, inoculated in the culture vessel, and then left to stand in a CO₂ incubator. No special culture vessel, for example, a culture vessel coated with extracellular matrix such as collagen, is required. Any material or shape may be used for the culture vessel as long as 3T3 fibroblasts, for example, can adhere to and proliferate in the culture vessel. Any culture vessel for adhesive cell which are commercially available such as flask, petri dish, roller bottle, well plate or tray, or any carriers such as conventional synthetic polymer membrane, film or plate, or biopolymer membrane, film or microbeads may be used, which can greatly reduce the process costs when compared to any conventional methods.